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Novel targets and clinically relevant models for ovarian cancer

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Chapter 1

General introduction

Introduction

Due to late presentation of symptoms, ovarian cancer is diagnosed at an advanced stage in most patients (1). Current standard of care for patients with advanced stage ovarian cancer consists of surgical debulking combined with paclitaxel and carboplatin for 6 cycles (2). Despite often aggressive surgery and high initial response rates, the majority of patients will relapse, with the length of the progression-free interval being a direct predictor of sensitivity to second-line platinum therapy (3-4). As a result, survival rates have not improved significantly over the last decades, with a 5-year survival rate of 19-28% in advanced stage disease (1).

A big leap in achieving upfront selection of ovarian cancer patients is the radical change in the definition of epithelial ovarian cancer, which is no longer conceived as one entity. It is now widely accepted that histological subtypes of ovarian cancer, i.e. high-grade serous, low-grade serous, endometrioid, mucinous and clear cell carcinoma, are derived via different pathways of tumorigenesis, divided in low-grade and high-grade pathways (5). Of these subtypes, high-grade serous ovarian cancer (HGSOC) is characterised by an almost ubiquitous presence of *TP53* mutations (6). In addition in ~20% of the HGSOC cases, a mutation in *BRCA1* or *BRCA2* is found, predisposing these women to hereditary breast and ovarian cancer. Remarkably, hardly any other gene mutations are found in HGSOC (6). In striking contrast, a remarkable degree of copy number aberrations and genomic instability is present, suggesting disruption of DNA repair pathways during early tumour development (7).

The different histological subtypes are known for their distinct platinum-sensitivity. The majority of HGSOC patients respond to first-line platinum-based chemotherapy, whereas clear cell, mucinous and low-grade serous tumours are reported to be more resistant (8-9). Especially HGSOC patients

harbouring a *BRCA1* or *BRCA2* mutation are described to be sensitive towards platinum-based chemotherapy with a significant better response rate and longer survival (10). *BRCA1* and *BRCA2* are both important players in the error-free homologues recombination (HR) repair pathway, which repairs double strand DNA breaks and stalled replication forks. A novel type of anti-cancer agents, Poly(ADP)ribose polymerase (PARP) inhibitors (PARPi), take advantage of defects in the HR repair pathway. By inhibiting the repair of single strand DNA breaks in a HR deficient background, treatment with PARPi results in double strand breaks, thus causing so-called "synthetic lethality". In *BRCA1/2* mutation carriers these agents have already shown promising results (11-12). Inhibitors of other DNA damage repair pathway components are increasingly under investigation for their potential to enhance effectiveness of current standard cancer treatments such as radiotherapy and chemotherapy (13).

Furthermore, targeting the anti-immune response of the tumour has recently gained much interest, for instance via blocking immune checkpoints such as programmed death-ligand 1 (PDL-1) (14). For quite some time, pro-apoptotic members of the tumour necrosis factor (TNF) family have been studied in ovarian cancer, for instance tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) and its receptors. One of the other TNF family members currently under investigation is CD70, ligand of the co-stimulatory TNF receptor superfamily member CD27 and transiently expressed on antigen-activated T and B-lymphocytes. Interestingly, in haematological cancers and in several solid cancers like melanoma, glioblastoma, renal cell-, pancreatic-, lung- and ovarian cancer, aberrant CD70 expression has been reported (15). As the role of CD70 in ovarian cancer has hardly been described and its importance is yet unknown, the characterisation of CD70 is warranted to investigate its function and its potential as a therapeutic target in ovarian cancer.

In the search for new targets and biomarkers, patient-tailored therapy, based on a patient's individual tumour characteristics, is widely considered as the next step in improving outcome, decreasing side effects and overcoming resistance (16). However, given the intertumoral, but also intratumoral heterogeneity of HGSOC, only subsets of patients will be likely to benefit from targeted therapies. Therefore, more representative experimental models are an important step in improving bench-to-bedside translation (17). The aim of this thesis is to obtain a better understanding of factors determining response to therapy in HGSOC patients by integrating multiple *in vitro* and newly established *in vivo* models. This thesis describes preclinical work on predictive biomarkers in the DNA damage response pathway as well as in the TNF family pathway, suitable for upfront selection of patients and as targets for innovative therapy. Furthermore, the development of an ovarian cancer patient-derived xenograft mouse model is extensively described, as well as the possible translational value of this model.

Outline of the thesis

In the past decades much progress has been made in developing representative ovarian cancer models using both *in vitro* and *in vivo* approaches that mimic ovarian cancer pathogenesis, tumour microenvironment, angiogenesis and therapy response (18). In **Chapter 2**, we review recently developed preclinical ovarian cancer models suitable for investigating response to platinum-based chemotherapy and biomarker-based personalised strategies to overcome platinum-based chemo-resistance. Finally, as an example of integration of several of these models, we discuss current studies on PARPi in *BRCA1/2*-mutation carriers and sporadic ovarian cancer patients displaying a so-called BRCAness phenotype.

The effectiveness of conventional DNA damaging chemotherapeutics is limited by DNA damage checkpoints and DNA repair mechanisms that

protect tumour cells from DNA damage-induced cell death. As part of the DNA damage response (DDR), the Ataxia Telangiectasia Mutated (ATM) signalling axis has drawn attention as a possible new target in enhancing the cytotoxic effectiveness of radiotherapy and chemotherapy. In **Chapter 3**, we immunohistochemically analyse the expression of several downstream targets in the ATM signalling pathway, using tumour tissue from a well-defined subset of advanced stage HGSOC patients. To explore the cell biological basis for high Chk2 expression being related to a good response to therapy, we subsequently study the effects of modulating Chk2 levels *in vitro* and investigate its effects on cisplatin sensitivity in two ovarian cancer cell lines.

Besides damage repair capacity, targeting anti-immune responses of tumours has also gained interest as a way of improving treatment outcomes. In **Chapter 4**, we evaluate CD70 expression levels in a large panel of ovarian cancer cell lines and investigate the relation of CD70 with cisplatin sensitivity. Furthermore, after having observed a CD70 splicing variant, CD70isoform (CD70iso), we investigate the cell surface expression of both CD70 variants. To study their functional significance, we transfect CD70-negative Chinese hamster ovary (CHO) cells with CD70wt or CD70iso and investigate their influence on peripheral blood mononuclear cell (PBMC) proliferation.

Patient-derived xenografts (PDXs) are increasingly considered as authentic preclinical models for studying ovarian cancer as they reflect heterogeneity of the original tumour and preserve response to therapy. However, using PDXs for preclinical cancer research demands proper storage of tumour material to facilitate logistics and to reduce the number of animals needed. In **Chapter 5**, we present our panel of ovarian cancer PDXs. Furthermore, we carefully compare two methods for biobanking of PDX tumour material: A fetal calf serum (FCS)-based “FCS/DMSO” freezing protocol and a low serum-based

"vitrification" protocol. We analyse and compare both methods in terms of take- and growth rate and resemblance to the parental tumour from the patient, using immunohistochemistry and copy number alterations.

A number of therapeutic targets are currently being explored for the treatment of ovarian cancer, such as vascular endothelial growth factor (VEGF) and insulin-like growth factor-1 receptor (IGF-1R). Development of non-invasive methods for detection of multiple tumour-related proteins in patients would certainly assist the selection of targeted therapies and monitoring early responses in individual patients. In **Chapter 6**, we test the feasibility of dual wavelength near-infrared fluorescence (NIRF) imaging in multiple ovarian cancer PDXs, using the monoclonal antibodies bevacizumab (anti-VEGF) and MAB391 (anti-IGF-1R) coupled to the NIRF dyes IRDye-800CW and IRDye-680RD, respectively. Furthermore, using this technique, we monitor the *in vivo* expression of VEGF and IGF-1R during treatment with cisplatin.

Finally, a summary of the results described in this thesis is presented in **Chapter 7**, along with a general discussion and future perspectives.

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